REMARKS/ARGUMENTS

Claims 12-19 and 62-79 are active in this application. Claims 1-11 and 20-61 have been canceled in view of the Examiner's final restriction requirement. Claim 62 is supported by the disclosure on pages 8-10 and is drawn to the elected subject matter. While Claims 63-79 are drawn to non-elected subject matter they have been added to depend on the elected claims so that these new claims can be rejoined once the Examiner has found the elected claims allowable (MPEP § 821.04).

No new matter is added.

The rejection of Claims 12-19 under 35 U.S.C. § 112, first paragraph is respectfully traversed.

The application provides not only one but numerous polynucleotides and amino acid sequences corresponding to the acid β-glucocerebrosidase ("GBA"). For example, the Examiner's attention is drawn to the disclosure on pages 7, 8 and 9 as well as the sequence listing filed with this application.

The present application also describes that these proteins can be treated to yield "highly phosphorylated GBA" which is clearly described on page 24, line 21 to page 26, line 24.

Highly phosphorylated as known in the art and described in the specification on page 15 relates to the presence of bis-phosphorylated oligosaccharides on the enzyme (see page 15, lines 3-11). These bis-phosphorylated oligosaccharide modified enzymes thus "have a greater affinity for the M6P receptor and are therefore more efficiently taken into the cell by plasma membrane receptors. These bis-phosphorylated oligosaccharide are known as illustrated in the attached Figures 7.5 and 7.6 from "Essentials of Glycobiology" Ajit Varki et al. (Eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1999. See also the

page titled "Symbolic Representations of Common Monosaccharides and Linkages" also from this text book.

Withdrawal of this ground of rejection is requested.

The rejection of Claims 12-19 under 35 U.S.C. § 112, second paragraph is respectfully traversed.

The essential inquiry pertaining to the requirement under 35 U.S.C. § 112, second paragraph is whether the claims set out and circumscribe a particular subject matter with a reasonable degree of clarity and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:

(A) The content of the particular application disclosure;

(B) The teachings of the prior art; and

(C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. See MPEP § 2173.02

As clearly set forth on page 15 of the specification, and further discussed above, the term "highly phosphorylated GBA" is defined. Therefore, one can unequivocally ascertain the standard of highly phosphorylated. In a similar way, it logically follows that if something is "not highly phosphorylated" it does not meat the standard or have the requisite degree of modification as an enzyme that is "highly phosphorylated."

Withdrawal of this ground of rejection is requested.

The rejection of Claims 12-19 under the doctrine of obviousness-type double patenting over Claims 9-12 of U.S. Patent No. 6,534,300 is addressed by the Terminal Disclaimer filed herewith.

The objection to Claims 12-19 has been addressed by amendment.

Applicant also requests that this application now be passed on to issuance.

Respectfully submitted,

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Symbolic Representations of Common Monosaccharides and Linkages

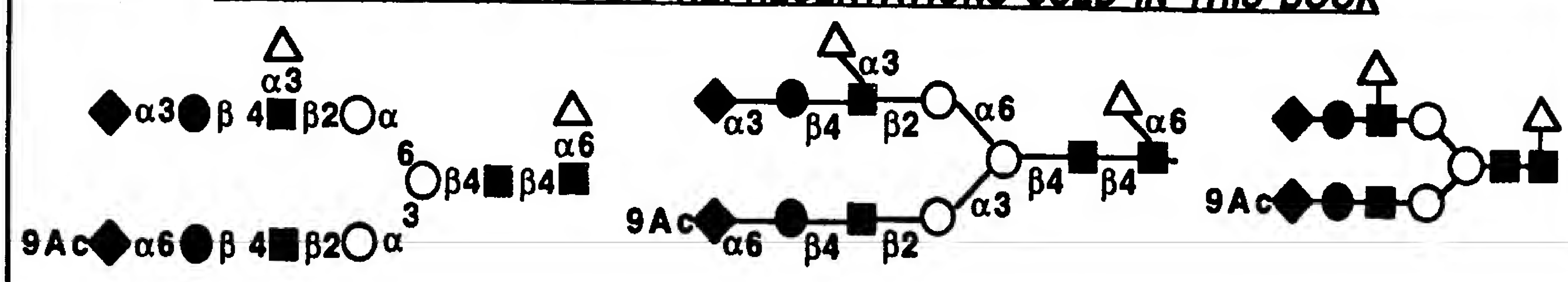
▲ = Glucose (Glc)
○ = Mannose (Man)
● = Galactose (Gal)
■ = N-acetylglucosamine (GlcNAc)
□ = N-acetylgalactosamine (GalNAc)
◩ = N-acetylhexosamine, unspecified (HexNAc)

◐ Hexose, unspecified (Hex)

△ = Fucose (Fuc)
▽ = Xylose (Xyl)
◆ = Sialic acid, unspecified (Sia)
⬘ = Glucuronic acid (GlcA)
⬙ = Iduronic acid (IdoA)
◇ = Uronic acid, unspecified (HexA)

Ac = O-acetyl   P = Phosphate   S = O-Sulfate   NS = N-Sulfate NH_2 = free amino group

EXAMPLES OF SYMBOLIC REPRESENTATIONS USED IN THIS BOOK



SIMPLIFIED TRADITIONAL REPRESENTATION

Fucα
3
Siaα3Galβ4GlcNAcβ2Manα
6
Manβ4GlcNAcβ4GlcNAc~
3
9Ac-Siaα6Galβ4GlcNAcβ2Manα

Fucα
6

FULL TRADITIONAL REPRESENTATION

Fuc*p*α1
3
Sia*p*α2-3Gal*p*β1-4GlcNAc*p*β1-2Man*p*α1
6
Man*p*β1-4GlcNAc*p*β1-4GlcNAc*p*~
3
9OAc-Sia*p*α2-6Gal*p*β1-4GlcNAc*p*β1-2Man*p*α1

Fuc*p*α1
6

The symbolic representation system shown here is used throughout this book. The example used is a typical branched "biantennary" N-glycan with two kinds of outer termini. The monosaccharides assigned the symbols are those most commonly found in higher animal glycoconjugates.

Unless otherwise indicated:

- all monosaccharides are assumed to be in the D-configuration, except for L-Fucose and L-Iduronic Acid.
- all glycosidically-linked monosaccharides are assumed to be in the pyranose *(p)* form (six-membered ring).
- all glycosidic linkages are assumed to originate from the C1 hydroxyl group except for sialic acids, which are linked from the C2 hydroxyl.

Essentials of Glycobiology

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Front Cover (printed hardcover): A ribbon diagram of the bovine cation-dependent Man-6-P receptor (CD-MPR). The two monomers (purple ribbon and blue ribbon) of the dimer as well as the ligand, Man-6-P (gold ball-and-stick model), are shown. (Modified, with permission, from Roberts et al., *Cell 93:* 639–648 [1998], Figure 4a.)

Back Cover (printed hardcover) High-mannose-type N-glycans can be processed down a variety of biosynthetic pathways, two of which are shown, using the symbol nomenclature recommended in this book. The diphosphorylated glycan shown is the optimal ligand for the mannose 6-phosphate receptors (see front cover).

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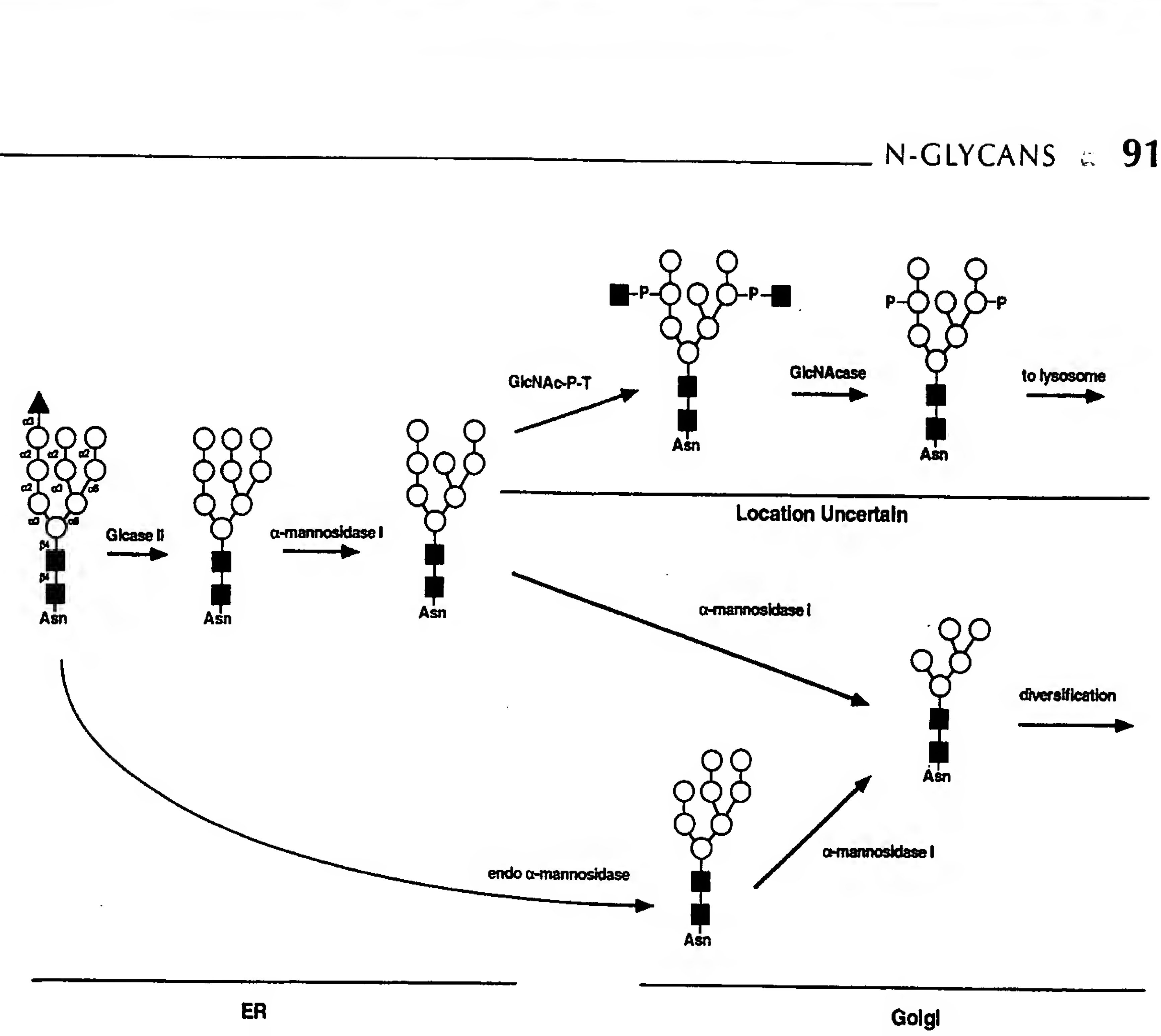

FIGURE 7.5. Processing of vertebrate N-linked oligosaccharides in the ER and Golgi. Most N-glycan processing takes place through the action of an ER-localized α-mannosidase I followed by a Golgi-resident isozyme. Some that have escaped into the Golgi with a $Glc_1Man_5GlcNAc_2$-Asn structure are acted upon by an endo-α-mannosidase. Glycoproteins bound for the lysosome are modified by a GlcNAc phosphotransferase (GlcNAc-PT) and subsequently a GlcNAcase to carry the Man-6-P signal.

the ER by removal of an α1-2-linked mannose on the middle chain. Next, and in contrast with vertebrate cells, yeast add mannose to this structure to produce high-mannose N-glycans with up to 15 mannose residues. In vertebrates, cell surface and secreted high-mannose N-glycans (Man_9 and smaller) can normally be found, generally at low levels, as most often processing has resulted in a $Man_5GlcNAc_2$-Asn glycan that becomes a substrate in the Golgi for the diversification of extracellular N-glycans (see below).

An exception to the above processing reactions exists as the result of a unique endo-mannosidase in vertebrates that acts on glucosylated N-glycan precursors in the Golgi that have escaped glucosidase action in the ER. The product of the endo-mannosidase reaction is $Man_{8\text{-}3}GlcNAc_2$-Asn, and this is the enzyme that may account for the inability of glucosidase inhibitors to completely block the production of further processing events in N-glycan diversification.

THE DIVERSIFICATION OF N-GLYCANS (35–42)

An understanding of substrate specificities regarding the glycosyltransferases and glycosidases acting in N-glycan biosynthesis has been obtained during the last two decades by analyses of lectin-resistant cell lines deficient in specific glycosyltransferases and glycosidases, as well as from the use of cell-free systems that process N-glycans in vitro. These specificities involve the oligosaccharide moieties from the previous enzymatic step. The processed high-mannose $Man_5GlcNAc_2$-Asn N-glycan serves as a substrate for the diversification of N-glycans in the Golgi. Extracellular N-glycans in vertebrates exist as high-mannose, hybrid, or complex subtypes (Figure 7.6). Hybrid structures are defined as those

with both substituted (GlcNAc linkage) and unsubstituted mannose residues. Complex N-glycans refer to those in which both the α3- and α6-linked mannose residues are substituted with GlcNAc moieties. When total N-glycans are analyzed from various cells, most vertebrate extracellular N-glycans are found to be of the complex subtype.

The first enzyme needed in building diverse N-glycan structures is termed GlcNAcT-I and is encoded by a single gene in mammals (*Mgat1*). GlcNAcT-I adds a GlcNAc in β1-2 linkage to produce a hybrid N-glycan structure that is the substrate for α-mannosidase II activity. α-Mannosidase II acts specifically on the $GlcNAc_1Man_5GlcNAc_2$-Asn hybrid N-glycan in the medial Golgi to remove the α1-3-linked and α1-6-linked mannose residues as depicted (Figure 7.6). The resulting processed hybrid N-glycan product ($GlcNAc_1Man_3GlcNAc_2$-Asn) is the specific substrate for the *Mgat2*-encoded enzyme GlcNAcT-II that catalyzes the conversion of hybrid to complex N-glycans. It was initially thought that the route to complex N-glycans always required α-mannosidase II processing; however, studies of α-mannosidase-II-deficient mice have indicated the presence of an alternate pathway independent of α-mannosidase II function. The candidate enzyme in this alternate pathway is a distinct α-mannosidase found enriched in Golgi fractions (termed α-mannosidase III) that acts on the processed high-mannose N-glycan $Man_5GlcNAc_2$-Asn, producing $Man_3GlcNAc_2$-Asn. This latter N-glycan is also found to be an excellent substrate for GlcNAcT-I in vitro, yielding $GlcNAc_1Man_3GlcNAc_2$-Asn by this alternate route.

N-glycans of the hybrid and complex type may exist with two or more GlcNAc-bearing branches that are referred to as antennae. In forming multi-antennary N-glycan structures, GlcNAc residues may be added to the trimannosyl core by six different GlcNAc transferases (I–VI) (Figure 7.7). Up to five branches have been observed on N-glycans of some vertebrate glycoproteins. GlcNAcT-I and GlcNAcT-II must both act to produce a

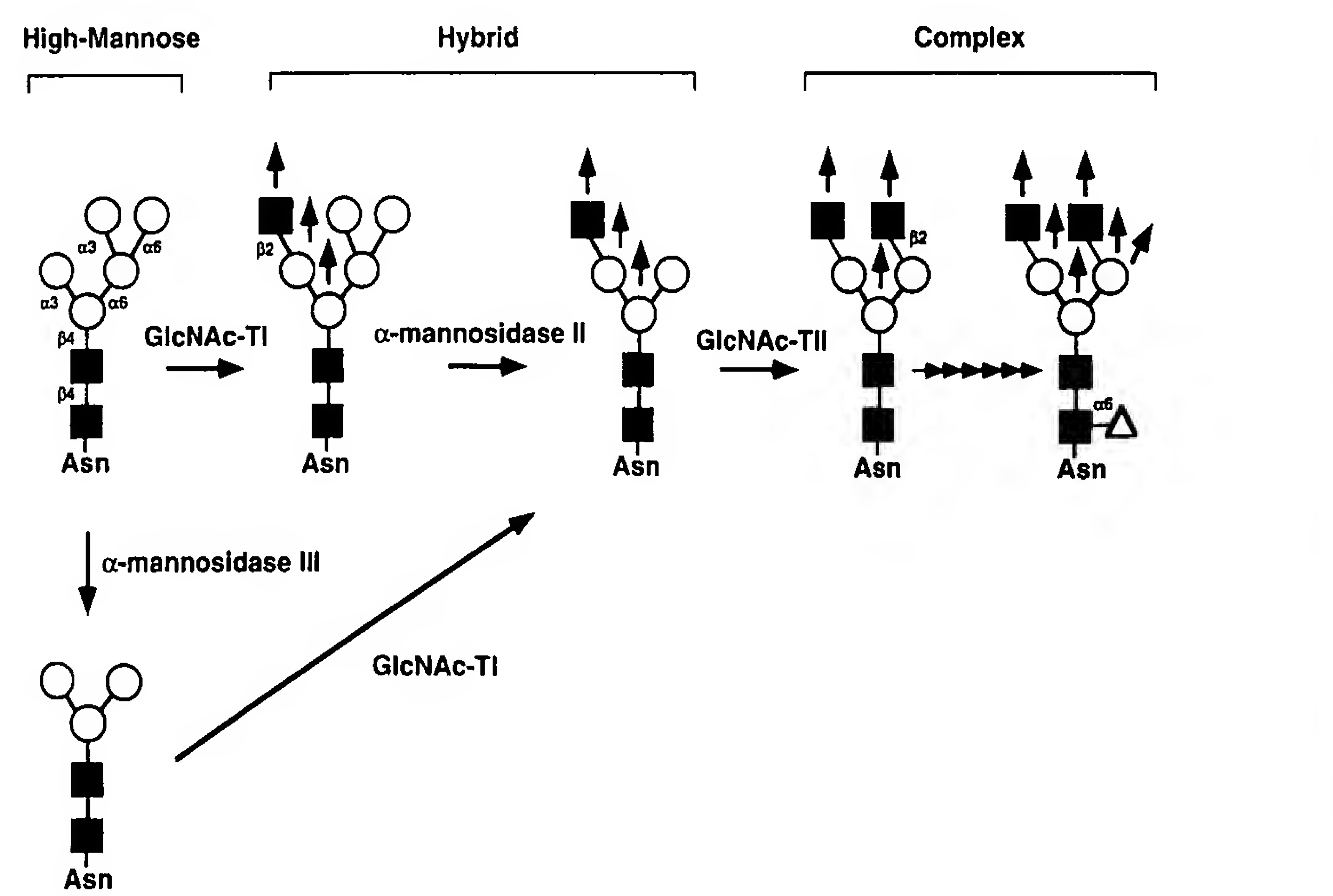

FIGURE 7.6. Vertebrate N-glycan diversification in the Golgi as shown generates three N-glycan subtypes: high-mannose, hybrid, and complex. Most secreted and cell surface N-glycans are of the complex type and are generated by one of two possible routes. α1-6 fucose can be added earlier than indicated. The vertical arrows depict locations of branch formation in N-glycan diversification, not all of which occur on any single N-glycan.

Dermatan Sulfate A modified form of chondroitin sulfate in which a portion of the β-glucuronate residues are epimerized to α-iduronates.

Dolichol A polyisoprenoid lipid carrier utilized during the assembly of N-glycans and GPI anchors.

β-Elimination Base-catalyzed, nonhydrolytic cleavage of an O-linked glycan attached to the hydroxyl moiety of a serine or threonine residue within a protein or peptide.

Endoglycosidase An enzyme that catalyzes the cleavage of an internal glycosidic linkage in an oligosaccharide or polysaccharide.

Endotoxin *See* Lipopolysaccharide.

Epimerase An enzyme that catalyzes racemization of a chiral center in a sugar.

Epimers Two isomeric monosaccharides differing only in the configuration of a single chiral carbon. For example, mannose is the C-2 epimer of glucose.

Exoglycosidase An enzyme that cleaves a monosaccharide from the outer (nonreducing) end of an oligosaccharide, polysaccharide, or glycoconjugate.

Exotoxins Heat-labile, proteinaceous toxins secreted by bacteria that cause illness.

Extracellular Matrix A complex array of secreted molecules including glycoproteins, proteoglycans, and/or polysaccharides and structural proteins. In plants, the extracellular matrix is also referred to as the cell wall.

Furanose Five-membered (four carbons and one oxygen, i.e., an oxygen heterocycle) ring form of a monosaccharide named after the structural similarity to the compound furan.

Galectins S-type (sulfhydryl-dependent) β-galactoside-binding lectins, usually occurring in a soluble form, expressed by a wide variety of animal cell types and distinguishable by distinguishable by the amino acid sequence of their carbohydrate recognition domains.

Ganglioside Anionic glycosphingolipid containing one or more residues of sialic acid.

Glycan A generic term for any sugar or assembly of sugars, in free form or attached to another molecule, used interchangeably in this book with saccharide or carbohydrate.

Glycation The nonenzymatic, chemical modification of proteins by addition of carbohydrate, usually through a Schiff-base reaction with the amino group of the side chain of lysine and subsequent Amadori rearrangement to give a stable conjugate. Not to be confused with (enzymatic) glycosylation.

Glycobiology Study of the structure, chemistry, biosynthesis, and biological functions of glycans and their derivatives.

Glycocalyx The cell coat structure consisting of glycans and glycoconjugates surrounding animal cells that is seen as an electron-dense layer by electron microscopy.

Glycoconjugate A molecule in which one or more glycan units are covalently linked to a noncarbohydrate entity.

Glycoforms Different molecular forms of a glycoprotein, resulting from variable glycan structure and/or glycan attachment site occupancy.

Glycolipid General term denoting a molecule containing a saccharide linked to a lipid aglycone. In higher organisms, most glycolipids are glycosphingolipids, but glyceroglycolipids and other types exist.

Glycomimetics Noncarbohydrate compounds that mimic the properties of saccharides.

Glycopeptide A peptide having one or more covalently attached glycan units.

Glycophospholipid Anchor (Glycosylphosphatidylinositol, GPI Anchor) A membrane anchor consisting of a glycan bridge between phosphatidylinositol and a phosphoethanolamine in amide linkage to the carboxyl terminus of a protein.

Glycoprotein A protein with one or more covalently bound glycans.

Glycosaminoglycans Polysaccharide side-chains of proteoglycans or free complex polysaccharides composed of linear disaccharide repeating units, each composed of a hex-